In the European Patent Office

European Patent No. 0712931

in the name of Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo

Declaration

I, Haruki Okamura, declare and say as follows:

1. I am a citizen of Japan residing at Nakahozumi, 2-12-32, Ibaraki-shi, Osaka, Japan.

2. I have graduated from Osaka University, Faculty of Science (Biology) in 1970 and received a doctorate of Science at Osaka University in 1976.

3. I have been working for Hyogo College of Medicine since 1976. I am the professor, Laboratory of Host Defenses, Institute for Advanced Medical Science, Hyogo College Medicine.

4. I have been engaged in research in the field of microorganisms and cytokines, particularly, interleukin-18 (IL-18). A true copy of my curriculum vitae is attached hereto.

5. I am an inventor on the above-identified patent. I also am a named author for D1 and D2 and D4.

6. I have read and am thoroughly familiar with the specification relating to the above-identified patent. Further, I have read and am thoroughly familiar with the content of the documents referenced as D1 and D2 in the statement of opposition filed at the European Patent Office by Centocor Inc., as well as the content of the statement *per se*. Also, I have read and am thoroughly familiar with the content of the document referenced as D4 cited in the submissions filed at the European Patent Office by Centocor Inc. on 7th January 2003.

7. D1 and D2 both are concerned with a murine "factor" that stimulates gamma interferon production. In these documents, emphasis is placed on the elucidation of the regulatory mechanism of IFN-γ production (D1 column 1 line 5 to 7 and D2 column 1 line 1 to column 2 line 13).

8. In D1, an "unknown" IFN-γ inducing factor originating from mice serum is referred to. Mere presence of the factor is demonstrated. There is no molecular characterisation of the factor and it is indicated that this will need to be clarified (see page 594, second full paragraph of column 1). D2 represents a development of the work described in D1. Again, a factor originating from mice spleen which stimulates IFN-γ production is referred to. The factor is said to be purified in D2 but remains unidentified (see page 68 line 12, column 2). It is stated that details of the molecule (factor) such as its terminal amino acid sequence or amino acid composition remain to be elucidated (see page 69 lines 8 to 12). We were unable to obtain a sufficient amount for these purposes. One therefore could not even infer from D2 whether the factor was a single molecule or a mixture of molecules. Furthermore, since we failed, one could not be sure whether the factor *per se* was such that it allowed isolation and characterisation.

9. The only information available to the skilled person from D1 and D2 is that a so-called "factor" of mouse origin exists, which is capable of stimulating IFN-γ production. In terms of providing a useful starting point for making the present invention; to provide a new substance which induces IFN-γ production, this extremely limited information on its own is of no practical use.

10. Further, since the factor of D1 had (i) IFN-γ inducibility, (ii) natural killer (NK)/LAK inducibility, (iii) DNA systhesis promoting activity, (iv) molecular weight of about 70,000 on gel filtration, and since the factor was (v) inactivated by heating at 80°C or by treating with protease, we speculated that the factor could possibly be natural killer stimulatory factor (NKSF)/interleukin-12 (IL-12).

11. Also, based on our finding that the apparent molecular weight of the active form of NKSF/interleukin-12 (IL-12); its IFN-γ inducibility; and the synergy with interleukin-2 (IL-2), anti-CD3 MAb, or the dynamics against mitogenic lectin were all similar to those of the factor of D2 (see page 69 left column, lines 25 to 28), we speculated that the factor of D2 could possibly be NKSF/interleukin-12 (IL-12) similarly as the factor of D1.

12. In addition, D4, published after the earliest priority date of the above-identified patent discloses a murine INF-γ inducing factor (murine "IGIF") as a new cytokine. The fact that

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the factor of D4 was reported as a new substance in "*Nature*", *INTERNATION WEEKLY*

JOURNAL OF SCIENCE, one of the famous academic journals in the field of science, indicates that the factor of D4 is not the same as the factors of D1 and D2.

13. When devising and embarking on the experiments that led to the present invention it was not obvious to me to try an approach, route or method with a reasonable expectation of success.

14. Our experiments were to provide a new substance which induces IFN-γ production . We decided to try to find out the DNA of a human IFN-γ production inducing factor and then to utilise gene recombinant technology to obtain the human IFN-γ production inducing polypeptide. We did not know whether a human IFN-γ production inducing factor even existed. When we embarked on these experiments, whilst we hoped to succeed, we had no ability to predict rationally that our project would reach a successful conclusion. This is because the experimental protocol that eventually proved to be successful was arrived at only by the good luck and inventiveness of those involved.

15. To suggest that arriving at our polypeptide merely involved purifying and sequencing the naturally occurring factor in D1 or D2 is completely incorrect. First and foremost, this is because the factor of D1 and D2 is of murine origin. In complete contrast, our polypeptide is of human origin. Our polypeptide therefore has a different specificity to the murine factor of D1 and D2.

16. Secondly, to sequence the factor of D1 or D2 one must have a sufficient amount. A sufficient amount could not be obtained by following the methodology in D1 or D2. In fact I note that we acknowledged this on page 69 column 1 lines 11 to 12 of D2.

17. To make our invention we decided to use recombinant gene technology. In order to utilise recombinant gene technology to obtain our polypeptide, we required mRNA of cells which produced human IFN-γ production inducing factor. D1 and D2 teach to assay mice spleen cells for murine IFN-γ production inducing factor. We however took a different approach.

18. We decided to select cells that would give us sufficient murine IFN-γ production inducing factor. We had no guidance or knowledge to help us make this selection. However,

one of the inventors in our team by chance noticed that a mouse in whose serum IFN-γ production inducing factor was found, was in the state of fulminant hepatitis. Based on this observation, the inventor made a guess that liver cells produce IFN-γ production inducing factor. At this moment, we had no guarantee that this guess was correct and, certainly, we had no way of predicting rationally that the liver cells would allow us to obtain a sufficient amount of the factor for our experiment. In fact, we were lucky and using the liver cells worked.

19. Having chosen mouse liver cells, the precise steps we took when putting into practice our theoretical experimental protocol played a further part in us reaching a successful conclusion. A particular combination of purification techniques was used in order to prepare the purified protein from the mouse liver cells. The particular combination of purification techniques we used and which were successful can in no way be considered together to be a standard purification method.

20. We then fortunately selected peptide fragments A and B from all peptide fragments eluted, as evidenced in Figure 1 of the above-identified patent. We had to select a part of one fragment on which to base a probe. We then had to select transformants from the cDNA library which we considered to strongly hybridise to the probe. We then selected SEQ ID NO:3 as a probe for human polypeptides and selected only phage DNA clones that we considered to have strong hybridisation to the probe. If we had not devised a suitable purification method for preparing the purified protein from mouse liver cells and if we had not taken the correct decisions in relation to the peptide fragments and probes used during our experiments, we would not have reached a successful conclusion.

21. In conclusion, it was not obvious to us when embarking on our experiments what particular approach we should use to obtain our polypeptide, particularly because we needed to find suitable cells which produced IFN-γ production inducing factor and we had no guidance or knowledge to help us make this selection.

22. Furthermore, having selected mouse liver cells from which to purify IFN-production inducing factor, we had no reasonable expectation of succeeding in reaching a successful conclusion because we did not know for sure that the mouse liver cells in fact produced the IFN-γ production inducing factor. Further, we did not know whether these

cells would produce the factor in a sufficient amount. Still further, we could not know that we would be able to take the correct decisions along the way during our experiments to be able to predict rationally that we would reach a successful conclusion.

I declare that all the statements made herein of my knowledge are true and that all statement made on information and belief are believed to be true.

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DATE: 24th day of March, 2005

CURRICULUM VITAE

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EDUCATION:

1970	Graduated from Osaka University, Faculty of Science (Biology)
1976	Degree of Ph.D, Osaka University
1976	Scholarship Student, Institute for Microbial Diseases, Department of Measles, Osaka University

BRIEF CHRONOLOGY OF EMPLOYMENT:

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1997-1999	Associate Professor, Laboratory of Host Defenses, Institute for Advanced Medical Science, Hyogo College of Medicine
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